AD_____

Award Number: DAMD17-97-1-7021

TITLE: Mammary Gland Ontogeny and Neoplasia in Oxytocin Deficient Mice

PRINCIPAL INVESTIGATOR: Janet A. Amico, M.D.

CONTRACTING ORGANIZATION: University of Pittsburgh
Pittsburgh, Pennsylvania 15260

REPORT DATE: July 2001

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2001	Final (01 Jul 97 - 30 Jun 01)

4. TITLE AND SUBTITLE
Mammary Gland Ontogeny and Neoplasia in Oxytocin Deficient Mice

5. FUNDING NUMBERS
DAMD17-97-1-7021

6. AUTHOR(S)
Janet A. Amico, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Pittsburgh
Pittsburgh, Pennsylvania 15260

E-Mail: jamico+@pitt.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)*

14. SUBJECT TERMS
Breast Cancer

15. NUMBER OF PAGES
5

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Key Research Accomplishments:

Rationale:

The neurohypophysial peptide oxytocin (OT) is synthesized within the paraventricular and supraoptic nuclei (PVN and SON) of the hypothalamus and stored within the posterior pituitary gland . The mammary gland is one of the major targets for OT's effects. During lactation suckling by the young stimulates release of OT from the posterior pituitary gland into the peripheral circulation. OT attaches to its receptor on the mammary myoepithelial cell and elicits contraction and the ejection of milk , In addition to its contractile effects, OT has been reported to enhance myoepithelial cell differentiation and proliferation in murine mammary organotypic cultures .

The pattern of mammary gland differentiation and proliferation in the presence and absence of OT has not been tested *in vivo*. Mice in which the gene for OT has been deleted (OT -/-) provide a novel and direct means of assessing the effects of OT deficiency upon the mammary gland. OT null mice are fertile and deliver their progeny normally at term, but are unable to nurse their young . Although milk production occurs, milk ejection does not ( ). Careful study of the mammary histology and differentiation at sequential stages in the life span of the OT -/- mouse compared to its wild type cohort (OT +/+) has not yet been reported. In the present study we investigated the ontogeny and histology of mammary development in both parous and virgin OT -/- mice.

Methods:

Female OT (-/-) and OT (-/-) mice of C 57BL/6 background were used for these studies ( ). The OT -/- mice were generated by Dr Scott Young, NIMH , and breeding pairs were purchased from Jackson Laboratories (Bar Harbor,ME). Animals were bred and housed for this study in the viral free quarters of the University of Pittsburgh Animal Facility under a 12-h light, 12-h dark cycle (lights on at 0700 h). Mice were housed in standard suspended or shoebox cages in groups of up to five animals per cage with free access to food (standard rodent chow) and water. The studies were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh.

Both virgin and parous (one prior pregnancy) OT -/- and OT +/+ mice were sacrificed in groups of 5-6 at 6, 12, 18, 24 months of life, whereas only virgin mice were sacrificed at 3 months of life

Animals were killed rapidly by scissors decapitation. After killing, the left anterior mammary glands were dissected from each animal and processed for paraffin embedding.

Tissues were cut and subsequently stained with hematoxylin-eosin. Immunoperoxidase staining with antibodies to smooth muscle actin (specific for myoepithelial cells) and keratin (specific for epithelial cells) were used to identify cell types. Blood vessels were identified by an antibody to Factor VIII.

Histological analysis of mammary tissue was performed on serial sections under the light microscope by two independent investigators. The analysis focused upon ductal structures, lobule development and periductal capillaries. The rating scale was as follows: Lobule development: 1 = none; 2= rare (TDLU); 3= 1-2 TDLU; Periductal capillaries: 1= up to 1/ duct or lobule; up to 2-3/ duct or lobule; >4 / duct or lobule. Based upon the rating scale an overall rank score was assigned to each tissue sample.

Samples were analyzed by multivariate ANOVA using age, genotype and parity as variables. When ANOVA indicated significant differences among groups, pairwise comparisons between groups were made by *post hoc* Fisher's protected least significant differences (PLSD) test.

Reportable Outcomes:

1- No gross or microscopic tumors of the mammary gland were identified in either OT -/- or OT +/+ mice.

2 – The histologic pattern of duct formation, lobule development and capillary architecture was not different in the mammary glands of OT -/- versus OT +/+ mice.

Conclusions:

Despite *in vitro* reports that OT promotes myoepithelial cell differentiation and proliferation in murine mammary organotypic cultures, absence of the gene for OT does not affect either the ontogeny of mammary development or the prevalence of murine mammary tumors.